

WHAT IS CLAIMED IS:

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1. An ultracentrifuge tube comprising an upper centripetal region, a middle region and a lower centrifugal region wherein an inner diameter of said upper region is larger than an inner diameter of said middle region and wherein an inner diameter of said middle region is larger than an inner diameter of said lower region.
2. The ultracentrifuge tube of claim 1, wherein said middle region comprises one or more serrations.
3. The ultracentrifuge tube of claim 1, wherein one wall of said centrifuge tube is linearly continuous.
4. The ultracentrifuge tube of claim 1, wherein said inner diameter of said lower region is smaller than 0.25 inch.
5. The ultracentrifuge tube of claim 1, wherein said lower region is at least 5% of the total length of said tube.
6. The ultracentrifuge tube of claim 1, wherein the inner surfaces are polished by vapor polishing.
7. The ultracentrifuge tube of claim 1, in which the inner surfaces are coated with adhering polymer to prevent adsorption of biological particles.
8. The ultracentrifuge tube of claim 1, wherein said lower region has an inner diameter small enough to trap an air bubble between two layers of liquid such that the air bubble will keep said two layers of liquid separate so long as said centrifuge tube is at rest.
9. A bucket for holding a centrifuge tube wherein said bucket comprises an upper region and a lower region, and wherein said lower region has a smaller outer diameter than said upper region.

10. The bucket of claim 9, wherein said bucket comprises a third region wherein said third region attaches said bucket to a rotor.
11. A method for concentrating microorganisms from a biological sample, wherein said method comprises the steps of:
- (a) adding a sample containing microorganisms to an ultracentrifuge tube and
 - (b) centrifuging said sample in said tube to concentrate said microorganisms, said ultracentrifuge tube comprising an upper region, a middle region and a lower region wherein an inner diameter of said upper region is larger than an inner diameter of said middle region and wherein an inner diameter of said middle region is larger than an inner diameter of said lower region.
12. The method of claim 11, wherein density gradients are formed in said lower region of said tube.
13. The method of claim 11, which further comprises placing two or more layers of fluid into said lower region of said tube prior to addition of said sample, said layers being separated by air bubbles.
14. The method of claim 11, which further comprises placing two or more layers of fluid into said middle region of said tube prior to addition of said sample, said middle region layers separated by one or more disks inserted into said tube, said disks capable of keeping said layers of fluid separate prior to centrifugation and of floating upwards during centrifugation.
15. The method of claim 14, in which all fluid layers decrease in physical density from a centrifugal to a centripetal direction.
16. The method of claim 14, in which enzymes, reagents, dyes, or fixatives are present in discrete layers and do not sediment appreciably under the conditions of centrifugation employed.

17. The method of claim 14, in which gradient solutes are chosen so that microorganisms preferentially survive passage through reagent layers while contaminating particles are degraded or dissolved.

18. The method of claim 14, in which the reagent layers are chosen to selectively degrade known classes of microorganism while allowing others to sediment to the centripetal isopycnic banding gradient.

19. The method of claim 14, in which bacteria are readily distinguished from viruses.

20. The method of claim 14, wherein said disks are porous.

21. The method of claim 11, which further comprises adding fluorescent stain to said sample.

22. A method for measuring the amount of DNA or RNA in microorganisms, which comprises concentrating the microorganisms according to the method of claim 11 and analyzing the amount of DNA or RNA by flow fluorescence analysis or epifluorescence analysis.

23. A method for distinguishing between single-stranded DNA viruses, double-stranded DNA viruses and RNA viruses present in a biological sample containing viruses, which comprises contacting dyes which can distinguish between said viruses with viruses in said sample, concentrating said viruses according to the method of claim 13, and detecting the bound dyes in a band of virus, whereby the type of nucleic acid present in the viruses is determined.

24. The method of claim 23, wherein said dyes are added to said sample.

25. The method of claim 23, wherein such dyes are added to a layer of fluid added to the tube before the addition of said sample and said viruses contact said dyes during centrifugation.

26. The method of claim 23, wherein said dyes are fluorescent and said bound fluorescent dyes are detected by passing an exciting fluorescent light through said band of virus and

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determining a wavelength of peak intensity of emitted fluorescent light from said band of virus.

27. The method of claim 26, which further comprises removing unbound dyes from said tube prior to determining said wavelength of peak intensity.

28. The method of claim 23, wherein said bound dyes are detected by passing an exciting light through said band of virus and determining the spectral distribution of the emitted light.

29. A method for determining an infectious agent titre in a biological sample, which comprises measuring the intensity of emitted fluorescent light of claim 26.

30. A method for determining titre in a biological sample of known volume wherein said method comprises the steps of:

- (a) concentrating said microorganism according to the method of claim 11;
- (b) removing fluid from above said lower banding region;
- (c) overlaying remaining fluid with water or buffer less dense than fluid in said lower region;
- (d) inserting a capillary tube with an open bottom end into said centrifuge tube such that said open bottom end is above one or more microorganism bands;
- (e) drawing fluid through said open bottom end of said capillary tube such that said fluid being drawn through said capillary tube forms a stream of fluid which passes through a flow cell where it is analyzed;
- (f) adding water or buffer to said upper region of said centrifuge tube as fluid is withdrawn in step (e) or as needed to maintain water or buffer above any viral band;
- (g) moving said centrifuge tube relative to said capillary tube such that said capillary tube moves into said lower region of said centrifuge tube and through any viral band of microorganisms;
- (h) analyzing for microorganisms in said stream of fluid flowing through said flow cell to determine a number of microorganisms present; and
- (h) calculating a titre from the determined number of microorganisms and known volume of said biological sample.

31. The method of claim 30, further comprising pumping fluid into a sheath around said stream of fluid exiting from said capillary tube thereby diluting said stream prior to passing through said flow cell.
32. The method of claim 31, wherein said sheath of fluid is pumped at a rate slower than the rate at which fluid passes through said flow cell.
33. The method of claim 32, wherein the flow of each liquid is controlled by gas pressure in place of pumps.
34. The method of claim 30, wherein said microorganisms are at a concentration in said capillary tube less than one-half their concentration in a band of microorganisms in said lower region of said centrifuge tube.
35. A method of determining which microorganism is present in a biological sample which contains a microorganism, wherein said method comprises the steps of:
- (a) concentrating said microorganism according to the method of claim 11;
 - (b) recovering the microorganism in a concentrated form
 - (c) subjecting said microorganism to mass spectroscopy to measure the masses of individual proteins;
 - (d) determining a mass spectrum of said sizes of proteins;
 - (e) comparing said mass spectrum of proteins with mass spectra obtained using known microorganisms; and
 - (f) determining that the microorganism in said biological sample is the same as a known microorganism which yields a mass spectrum identical with the mass spectrum obtained for the microorganism from said biological sample.
36. The method of claim 35, wherein said mass spectrometry is matrix assisted laser desorption ionization time of flight mass spectrometry.
37. The method of claim 35, wherein said mass spectrometry is electrospray mass spectrometry.

38. The method of claim 35, wherein said proteins are enzymatically digested prior to obtaining a mass spectrum.

A 39. A method of determining which microorganism is present in a biological sample which contains a microorganism wherein said method comprises the steps of:

- (a) concentrating said microorganism according to the method of claim 11, to yield concentrated microorganism;
- (b) extracting nucleic acid from said concentrated microorganism;
- (c) incubating said nucleic acid with restriction enzymes to produce nucleic acid fragments;
- (d) staining said nucleic acid or nucleic acid fragments;
- (e) determining a pattern of sizes of said nucleic acid fragments; and
- (f) comparing said pattern of sizes with patterns of sizes of nucleic acids, digested with said restriction enzymes, obtained from known microorganisms,

wherein said microorganism in said biological sample is identified as a microorganism which has an identical restriction fragment pattern.

40. The method of claim 39, wherein said sizes of nucleic acid molecules or fragments thereof are determined using flow cytometry.

41. The method of claim 39, wherein said sizes of nucleic acid molecules or fragments thereof are determined by gel electrophoresis.

42. The method of claim 39, wherein said sizes of nucleic acid molecules or fragments thereof are determined by mass spectrometry.

43. The method of claim 39, wherein said size of nucleic acid molecules or fragments thereof are determined by optical mapping.

~~44. A method of determining the mass of a microorganism genome of a microorganism in a biological sample wherein said method comprises the steps of:~~

- ~~(a) concentrating said microorganism by the method of claim 11;~~

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~~(b) staining said microorganism genome;~~

~~(c) purifying said microorganism genome; and~~

~~(d) subjecting said microorganism genome to fluorescence flow cytometry.~~

45. The method of claim 44, wherein said microorganism genome is digested with restriction enzymes prior to step (d).

~~46. A method of identifying a microorganism in a biological sample, wherein said method comprises the steps of:~~

~~(a) concentrating said microorganism according to the method of claim 11; and~~

~~(b) incubating with antibodies specific for known microorganisms,~~

~~wherein if said antibodies bind to said concentrated microorganism then said microorganism is identified as the microorganism to which the antibodies are known to bind, by their fluorescence.~~

47. The method of claim 46, wherein said fluorescent antibodies are present in said upper region of said centrifuge tube during centrifugation of said biological sample, attach to the microorganism for which they are specific during incubation, cosediment and coband with said microorganism, and are detected by the fluorescence of said antibody-microorganism conjugate band.

48. The method of claim 47, wherein a plurality of species of antibody is present in said upper region of said centrifuge tube during centrifugation of said biological sample and wherein each species of antibody is labeled with a marker distinct from any marker on any other species of antibody present in said upper region.

49. The method of claim 46, in which the antibody microorganism complex has a banding density different from that of the free microorganism, thus allowing the presence of the complex to be detected.

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50. A method of separating layers in a centrifuge tube prior to centrifugation wherein fluid in said centrifuge tube comprises a first dense layer and a second less-dense layer, wherein said method comprises the steps of:
- (a) inserting said first dense layer into said tube;
 - (b) providing a means for separating the first and second layers; and
 - (c) inserting said second less-dense layer into said tube,
- wherein said centrifuge tube comprises an upper region, a middle region and a lower region, wherein an inner diameter of said upper region is larger than an inner diameter of said middle region and wherein an inner diameter of said middle region is larger than an inner diameter of said lower region.
51. The method of claim 50, wherein said means for separating said layers is an air bubble.
52. The method of claim 50, wherein said means for separating said layers is a porous disk and said porous disk is inserted on top of said first layer.
53. The method of claim 52, wherein said disk floats during centrifugation to a region above said second less-dense layer, thereby allowing said first dense layer to contact said second less-dense layer.
54. The method of claim 52, wherein said disk is made of sintered polyethylene or polypropylene.
55. The centrifuge tube of claim 1, wherein said centrifuge tube is prepared from materials such that said tube can be centrifuged at velocities high enough to band microorganisms in CsCl gradients without said centrifuge tube breaking.
56. The centrifuge tube of claim 55, wherein said tube is made of polycarbonate.
57. The centrifuge tube of claim 1, wherein said upper region, middle region, and said lower region have outer diameters equal to each other.

58. The centrifuge tube of claim 1, wherein said upper region has an outer diameter larger than an outer diameter of said lower region.
59. The method according to claim 11, wherein said centrifuge tube is supported by an adapter with inner dimensions contoured to match said centrifuge tube's outer dimensions.
60. The method according to claim 59, wherein said adapter is manufactured from polycarbonate or Delrin®.
61. A system for measuring fluorescence from a sample in a centrifuge tube wherein said system comprises:
- a centrifuge tube holder to hold a centrifuge tube in a vertical position;
 - a laser which produces a laser beam;
 - a filter for isolating light of one wavelength;
 - a filter through which passes light emitted by excited dye bound to said sample which has been banded in a centrifuge tube when said centrifuge tube is placed into said centrifuge tube holder; and
 - a detector which detects light passing through the filter of part (c).
62. The system of claim 61, comprising in addition
- goniometers onto which said centrifuge tube holder is mounted thereby enabling a centrifuge tube which is in said centrifuge tube holder to be oriented to match the vertical angle of the laser beam;
 - an X-Y movement to align the tubes with the laser beam in any X-Y direction;
 - a filter through which said laser beam passes; and
 - a mirror to deflect said laser beam through a centrifuge tube held in said centrifuge tube holder.
63. The system of claim 61, further comprising a computer.
64. The system of claim 63, further comprising a monitor.

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65. The system of claim 64, wherein said monitor is a cathode ray tube.
66. A system for measuring fluorescence from a sample in a centrifuge tube wherein said system comprises:
a holder for said centrifuge tube;
a light source to produce light which will pass through said sample; and
a detector to detect light which is emitted from said sample upon having light passed through it.
67. The system of claim 66, further comprising:
a condensing lens through which said light from said light source passes;
a filter through which said light from said light source passes;
an intensity equilibrators through which said light passes;
light pipes through which said light passes; and
two intensity equilibrators through which light leaving said light pipes passes.
68. The system of claim 67, further comprising:
an emission filter through which light emitted from said sample passes.
69. The system of claim 67, wherein said filter is replaced by a filter wheel comprising more than one filter.
70. The system of claim 67, further comprising:
a filter wheel comprising more than one emission filter wherein any one emission filter of said filter wheel can be placed between light emitted from said sample and said detector.
71. The system of claim 66, further comprising a computer.
72. The system of claim 71, further comprising a monitor.
73. The system of claim 72, wherein said monitor is a cathode ray tube.

74. A system for counting particles which are concentrated in a small volume, wherein said system comprises:
- a container in which said particles are concentrated;
 - a capillary tube;
 - a first pump and a second pump;
 - means for moving said container relative to said capillary tube;
 - a flow cell;
 - a light source; and
 - a detector.
75. The system of claim 74, wherein said first pump pumps fluid into a sheath around an upper end of said capillary.
76. The system of claim 75, wherein said second pump pumps fluid out of said flow cell.
77. The system of claim 76, wherein said second pump pumps more fluid in a given time period than said first pump.
78. The system of claim 77, wherein fluid is drawn through a lower end of said capillary tube, said fluid exits said upper end of said capillary tube where it is surrounded by fluid pumped from said first pump, and wherein said second pump pumps the mixture of fluid exiting from said upper end of said capillary tube and said fluid from said first pump through said flow cell.
79. The system of claim 78, wherein said light source produces a light beam which passes through said flow cell.
80. The system of claim 79, further comprising:
- a filter between said light source and said flow cell.
81. The system of claim 80, further comprising:
- a filter between said flow cell and said detector.

82. A method of determining the size of a genome of a microorganism in a biological sample, wherein said method comprises the steps of:

- (a) concentrating said microorganism by the method of claim 11, to produce concentrated microorganism;
- (b) extracting said genome from said concentrated microorganism to produce extracted nucleic acid;
- (c) immobilizing said extracted nucleic acid on a solid support;
- (d) staining said extracted nucleic acid; and
- (e) electronically imaging said extracted and stained nucleic acid on said solid support using an epifluorescence microscope, and
- (f) measuring the length of individual nucleic acid molecules.

83. A method for determining a restriction enzyme map of a microorganism, wherein said method comprises the steps of:

- (a) concentrating said microorganism by the method of claim 11, to produce concentrated microorganism;
- (b) extracting said genome from said concentrated microorganism to produce extracted nucleic acid;
- (c) staining said extracted nucleic acid;
- (d) immobilizing said extracted nucleic acid on a solid support to produce immobilized nucleic acid;
- (e) treating said immobilized nucleic acid with one or more restriction enzymes; and
- (f) determining the number of fragments of nucleic acid and the lengths of nucleic acid fragments produced.

84. A method for determining the identity of a microorganism in a biological sample, wherein said method comprises the steps of:

- (a) determining a restriction map according to the method of claim 83; and
- (b) comparing said restriction map to restriction maps of known microorganisms, wherein a match of restriction maps of said microorganism in said biological sample with a restriction map of a known microorganism identifies the microorganism of

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said biological sample as being that of said known microorganism with an identical restriction map as that of the microorganism of said biological sample.

85. ~~A method for distinguishing the type of infection between virus, mycoplasma, yeast and bacterial infections by determining the presence of a virus, mycoplasma, yeast or bacteria in a biological sample, which comprises the steps of:~~

- (a) ~~concentrating said microorganism by the method of claim 11, to produce concentrated microorganism;~~
- (b) ~~staining the microorganism with a fluorescent dye;~~
- (c) ~~measuring the amount of nucleic acid in the stained, concentrated microorganism, and~~
- (d) ~~comparing the amount of said nucleic acid to the known amount of nucleic acid for viruses, mycoplasmas, yeast and bacteria.~~

86. The method of claim 85, wherein the microorganism is stained during centrifugation.

87. The method of claim 85, wherein the microorganism is stained in the biological sample.

88. The method of claim 85, wherein the amount of nucleic acid is measured by flow cytometry.

89. The method of claim 85, wherein the amount of nucleic acid is measured by optical mapping.

90. The method of claim 29, in which the changes in the titre of an infectious agent are used to determine which known pharmacological agents are therapeutic, to evaluate the efficacy of new drugs in animal and human trials, and to choose between analogues of drugs in development.

91. The method of claim 29, in which the changes in the titre of an infectious agent are used to discover new antibiotics and other therapeutic agents.

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